

# High temperatures disrupt *Artemia franciscana* mating patterns and impact sexual selection intensity

Maria R. Santos<sup>a</sup>, Natividade Vieira<sup>b,c</sup>, Nuno M. Monteiro<sup>a,d,\*</sup>

<sup>a</sup> CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, rua Padre Armando Quintas, 4485-661 Vairão, Portugal

<sup>b</sup> Faculdade de Ciências da Universidade do Porto, rua do Campo Alegre, 4169-007 Porto, Portugal

<sup>c</sup> CIIMAR/CIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

<sup>d</sup> CEBIMED, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, rua Carlos da Maia 296, 4200-150 Porto, Portugal

## ARTICLE INFO

### Keywords:

Sexual selection  
Mating system  
Climate change  
Mate choice  
Competition  
Adaptation

## ABSTRACT

Temperature plays a critical role in survival and reproduction, especially in ectotherms. Therefore, it is important to understand the mechanisms influencing life history traits and reproductive behaviours in order to predict climate change impacts on species' occurrence and performance. Here, we used the crustacean *Artemia franciscana* to investigate the potential impacts of temperature on life history traits, mating patterns and intensity of sexual selection. We reared *A. franciscana* at three temperatures 20 °C, 25 °C, and 30 °C and measured life history traits such as growth, mortality or development of sexual traits. Our observations confirmed a clear link between life history traits and temperature, with advanced sexual maturity and increased mortality rates following temperature rises. Also, we found that mating is size assortative close to the ideal developmental temperature. Nevertheless, when temperatures deviate from the optimum, mating patterns were altered. Although selection intensity for females remained similar at all tested temperatures, as males preferentially mated with the larger females, size assortative mating disappeared at the highest temperature. Overall, our results highlight the potential for a temperature-dependent disruption of *A. franciscana* mating patterns. This disruption is especially pronounced under high temperatures as reproduction becomes progressively more random, thus entailing a relaxation of sexual selection intensity.

## 1. Introduction

Occurring at an exceptional rate, climate change represents an immense threat to global biodiversity (Bellard et al., 2012). Organisms in all ecosystems are increasingly challenged to adapt to the rapidly shifting conditions and evident alterations in natural populations' geographical distribution and phenology are being continually documented (Parmesan and Yohe, 2003; Hoffmann and Sgro, 2011). As temperature assumes a critical role in survival and reproduction, especially in ectotherms, one must unavoidably understand the mechanisms supporting current distributional patterns. This way, we will be able to better predict climate change impacts on species' occurrence and performance.

Populations that are becoming progressively closer to their upper thermal tolerance limits are especially susceptible to local extinctions (Somero, 2010) unless they disperse or adjust to the new conditions through plastic or genetic changes (Hoffmann and Sgro, 2011). However, when sexual reproduction is involved, the potential for local adaptation, under a climate change scenario, will surely depend on the

interplay between natural and sexual selection. Both of these processes are able to affect pre- and post-copulatory reproductive traits (Grazer and Martin, 2012). Although temperature can have strong effects on reproduction and, consequently, on population fitness, the influence of climate change on the intensity of sexual selection has received less than the deserved attention (Candolin and Heuschele, 2008). However, there are several reports that highlight the potential impacts of rising temperatures on the expression of sexual selection. For instance, in the wolf spider (*Pardosa astrigera*), courtship latency and duration, as well as copulatory duration declined with increasing temperatures (Xiauguo et al., 2009). In the worm pipefish (*Nerophis lumbriciformis*), populations closer to the species thermal tolerance limits showed enhanced expression of sexual traits and increased investment in reproduction (Monteiro and Lyons, 2012; Monteiro et al., 2017). A latitudinal cline in sexual selection pressure was also observed in medaka (*Oryzias latipes*) (Fujimoto et al., 2015). As temperature regimes can modify the costs and benefits arising from sexual selection, the potential rate of adaptation may ultimately be impacted. Although Candolin and Heuschele (2008) cautiously say that the role of sexual selection in extinction is

\* Corresponding author. CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, rua Padre Armando Quintas, 4485-661 Vairão, Portugal.  
E-mail address: [nm.monteiro@icloud.com](mailto:nm.monteiro@icloud.com) (N.M. Monteiro).

probably minor, they also point out that this subject is far from clear.

Here, we used the crustacean *Artemia franciscana* to investigate the potential impacts of temperature on the expression of the species mating system. We selected this species due to four main reasons: 1) available knowledge on the species' biology (Criel and Macrae, 2002), mating system (scramble-competitive polygamy; Allen et al., 2017) and sexual behavior (Tapia et al., 2015); 2) marked sexual dimorphism (e.g. males display strikingly enlarged secondary antennae used as clasping organs during mating), suggesting an active role of sexual selection (either through mate choice, intersexual competition, or both); 3) well-documented tolerance to a wide range of water temperatures (Lenz and Browne, 1991; Medina et al., 2007), as we were especially interested in observing reproductive changes despite high levels of phenotypic plasticity; and finally, 4) the need for additional information on the species potential for adaptation, as *A. franciscana* is an invasive species in the Iberian Peninsula (Amat et al., 2005; Rodrigues et al., 2012). We hypothesised that temperatures close to the species thermal tolerance limits would impact the expression of sexual characters, the mating system and, consequently, the intensity of sexual selection.

## 2. Methods

### 2.1. Origin and excystation

A single batch of *Artemia franciscana* (diploid, sexual) cysts, from San Francisco Bay (USA), were cultured for our experiments. *Artemia* were mass-hatched from cysts, according to Amat et al. (2005), in synthetic sea water (35 ppm), and fed daily with live microalgae (*Phaeodactylum tricornutum*). Nauplii were immediately transferred into nine (1L) experimental glass containers (density of  $\approx 11400$  nauplii per jar), that were then randomly assigned to the three temperature treatments described below. Seawater was changed ( $\approx 90\%$ ) every week and salinity adjustments were performed daily, when necessary. The individuals remained within the same jars up to sexual maturity.

### 2.2. Temperature treatments

The growth and reproductive performance of *Artemia* was tested at three distinct temperatures (20 °C, 25 °C and 30 °C), at a constant salinity of 35 ppm (synthetic seawater, Tropic Marin). These temperatures were selected based on the results from Browne et al. (1988) that showed *A. franciscana* to be well adapted to mid temperatures (24 °C) while still tolerating higher values (24–30 °C). For each temperature, one large glass aquaria served as a thermostatically controlled water bath (temperatures were maintained within  $\pm 1$  °C), housing three glass containers containing 900 mL of saltwater and an aeration system to maintain oxygenation. Water temperature and salinity were registered daily in each of the 9 jars. During the experiments, the natural photoperiod was approximately 14L:10D.

At least 10 individuals from each jar (30 individuals per temperature) were regularly photographed to record body length, up to sexual maturity. Nauplii were initially photographed under a Zeiss Axio Scope A1 microscope but, as individuals grew larger, we switched to a handheld USB digital microscope. Size measurements were first conducted in the software Zen (Zeiss) and then in ImageJ (version 1.50i). One of the 20 °C replicate jars was discarded early on due to a handling accident. As there were no differences in size among replicates (One-way ANOVA with size data from day 36; 20 °C:  $F(1,38) = 0.520$ ,  $P = 0.475$ ; 25 °C:  $F(2,57) = 0.859$ ,  $P = 0.429$ ; 30 °C:  $F(2,57) = 2.639$ ,  $P = 0.080$ ), we opted to pool data within temperatures.

Sexual maturity was determined, after the development of secondary sexual traits, by the observation of couples in riding position. These couples were removed from the jars to smaller individual containers (150 ml plastic cups) and, once the riding position ended, we individually photographed the male (to record body length, interorbital distance, and the length and width of the secondary antennae) and the

female (to record body length, interorbital distance and brood pouch area). Individuals were first anesthetized using chloroform saturated water prior to photographs (Amat, 1980). As the duration of the riding position can vary from days to hours (Amat et al., 2007; Anufrieva and Shadrin, 2014), we sometimes missed male-female interactions and later found females already carrying eggs in the brood pouch. These females were also photographed, as mentioned above, even though we had no phenotypic information on the mating male. The swimming velocity of adult individuals was analyzed in short videos of adult *A. franciscana* temporarily transferred to small Petri dishes (5 cm diameter).

### 2.3. Statistical analysis

We used the R package Growthcurver to compute *A. franciscana* growth curve metrics (e.g., growth rate:  $r$ ; carrying capacity:  $k$ ), from hatching up to day 37 (near the onset of sexual maturity at 30 °C), using the logistic equation (Sprouffske and Wagner, 2016). The developmental speed of selected traits was analyzed using Analysis of Covariance (ANCOVA), with body length used as covariate. The strength of selection on body size (Shuster and Wade, 2003) was assessed using the standardized selection differential for males and females ( $S_{\text{males}}$  and  $S_{\text{females}}$ ), in the three selected temperatures (selection differential divided by the standard phenotypic deviation of the trait, where the selection differential is the difference in the mean of the selected individuals in amplexus from the mean of the population).

The software Tracker (v 4.9.8) was used to calculate individual average swimming velocities, in males and females from the three selected temperatures. Individual velocity (cm/s) and body length of 78 individuals (33 females and 45 males) were registered and used to estimate the probability of observing size assortative mating due solely to swimming velocity (i.e. without mate choice directed to any other variable), at the three selected temperatures (20 °C:  $N = 20$ ; 25 °C:  $N = 28$ ; 30 °C:  $N = 30$ ). We simulated, in R, 1000 hypothetical populations per temperature, each comprising 25 couples whose individuals (males and females) derive from a replication, with replacement, of the actually measured individuals. Within each simulated population, we then selected from the randomly formed couples, those showing a difference in velocity between the sexes lower than 0.5 cm/s (average standard deviation of velocity in the measured groups  $\approx 0.491$  cm/s). If swimming speed was, hypothetically, the only character responsible for couple formation, then these couples formed by individuals with comparable speeds would have the largest probability of breeding. Within each selected couple, we then replaced individual swimming velocity for body length and looked for significant correlations between male and female size (i.e. a clear pattern of size assortative mating) within each simulated population. We assessed the probability (%) of finding non-random mating by dividing the number of theoretical populations found with size assortative mating by 1000, multiplied by 100. We replicated this procedure 10 consecutive times (overall, we conducted 10,000 simulations per temperature) to estimate average and standard deviation of our simulated probability estimates.

Statistical analyses were conducted in R, Statistica (v13) and GraphPad Prism (v6). For parametric tests, assumptions were met (e.g. homogeneity of variances) with the exception of the ANOVA on female body length (two factors: reproductive status and temperature). Nevertheless, as there was no significant correlation between averages and standard deviations, we proceeded with the analysis, as advocated by Lindman (1974). In ANOVAs and ANCOVAs, temperature was coded as a random factor.

## 3. Results

### 3.1. Temperature effects in growth, sexual maturity and life span

*Artemia* grew faster as temperature increased (Fig. 1), attaining

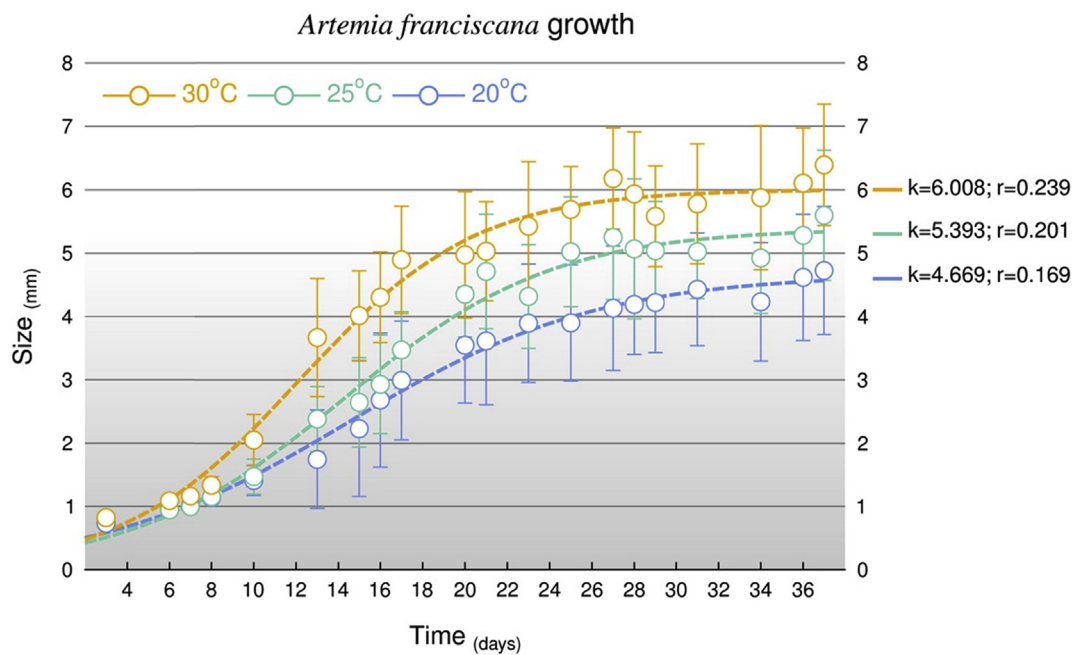


Fig. 1. Growth curves for *Artemia franciscana* raised at three distinct temperatures (20 °C, 25 °C and 30 °C), from hatching to the onset of maturity ( $k$  = carrying capacity;  $r$  = growth rate).

sexual maturity sooner at higher temperatures (30 °C: from day 36; 25 °C: from day 41; 20 °C: from day 65). Increased growth rates were accompanied by larger mortalities positively correlated with increasing water temperatures (determined at day 22:  $Y = 0.514x + 81.408$ ,  $R^2 = 0.732$ ,  $P < 0.01$ ).

Given the observed temperature-related changes in growth rate, we looked into the development of traits potentially able to enhance reproductive output, either directly (female brood pouch area, male antennae width and length) or indirectly (interorbital distance), looking for signals of distinct temperature-dependent trait development rates. Traits showed similar developmental rates at the three tested temperatures (ANCOVAs, size used as covariate: male secondary antennas length  $F(2,76) = 2.221$ ,  $P = 0.115$ ; male secondary antennas width  $F(2,76) = 0.526$ ,  $P = 0.593$ ; female brood sac area  $F(2,68) = 2.344$ ,  $P = 0.104$ ; male interorbital distance  $F(2,76) = 1.076$ ,  $P = 0.346$ ). The sole exception was female interorbital distance ( $F(2,75) = 3.802$ ,  $P < 0.05$ ) where, only at 20 °C, females showed a steeper interorbital distance increase with body growth (Newman-Keuls post-hoc test). Independently from temperature, larger females showed wider interorbital distances, larger brood sacs and larger egg-occupied areas (Fig. 2). Similarly, larger males showed wider inter-orbital distances, as well as wider and longer secondary antennas (see Fig. 2).

### 3.2. Temperature effects in the mating system

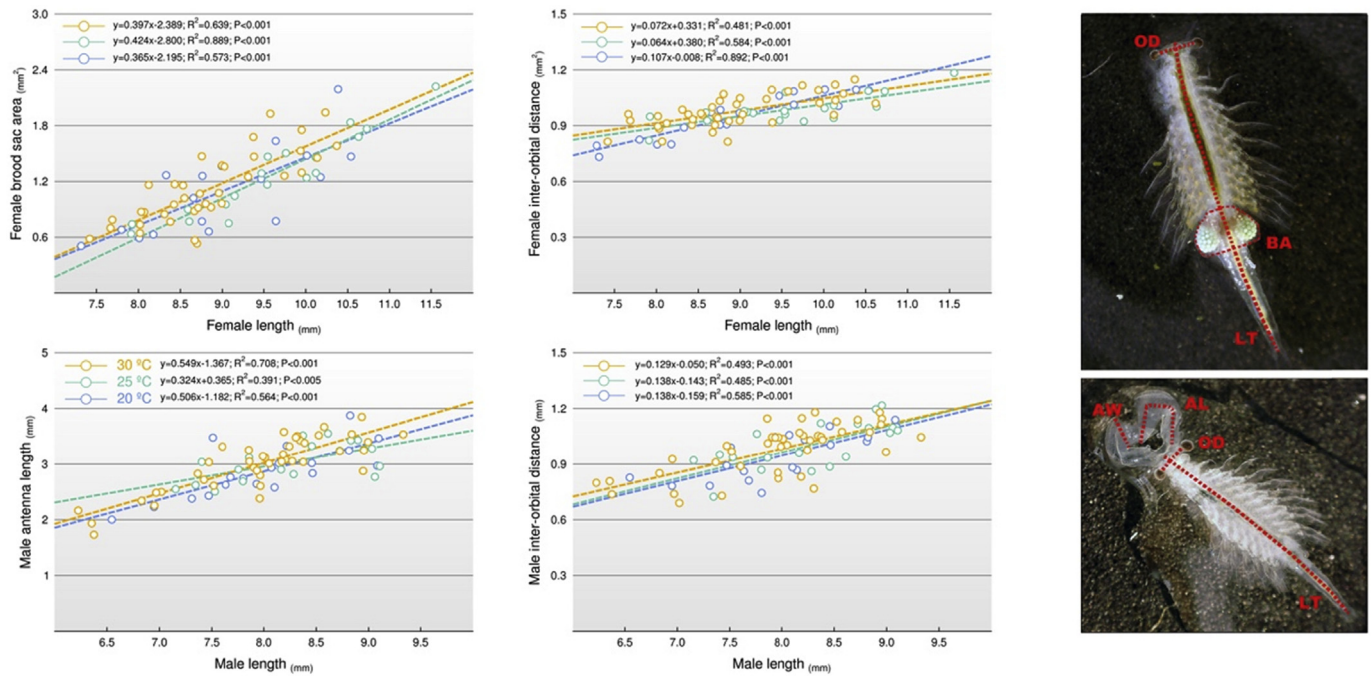
Looking at the body length of breeding individuals, we detected, at both 20 °C ( $y = 0.302x + 5.209$ ,  $R^2 = 0.279$ ,  $P < 0.05$ ) and 25 °C ( $y = 0.283x + 5.364$ ,  $R^2 = 0.230$ ,  $P < 0.05$ ), that mating was not random. At these temperatures, we observed size-assortative pairing (Fig. 3, bottom panels). Slopes ( $F(1,33) = 0.011$ ,  $P = 9.916$ ) and elevations ( $F(1,34) = 0.006$ ,  $P = 0.937$ ) of size assortative mating at 20 °C and 25 °C were similar. Contrastingly, at the highest tested temperature (30 °C), mating was clearly non-size-assortative ( $R^2 = 0.011$ ,  $P = 0.511$ ; Fig. 3, right bottom panel). Sexual size dimorphism, with females larger than males (20 °C: 84.21%; 25 °C: 88.88%; 30 °C: 82.5%), did not differ between temperature treatments (one-way ANOVA:  $F(2,76) = 1.615$ ,  $P = 0.206$ ) despite being higher at 25 °C ( $1.297 \pm 0.845$  mm; average  $\pm$  standard deviation), followed by the other two temperatures (20 °C:  $0.814 \pm 0.978$  mm; 30 °C:

$0.812 \pm 1.074$  mm).

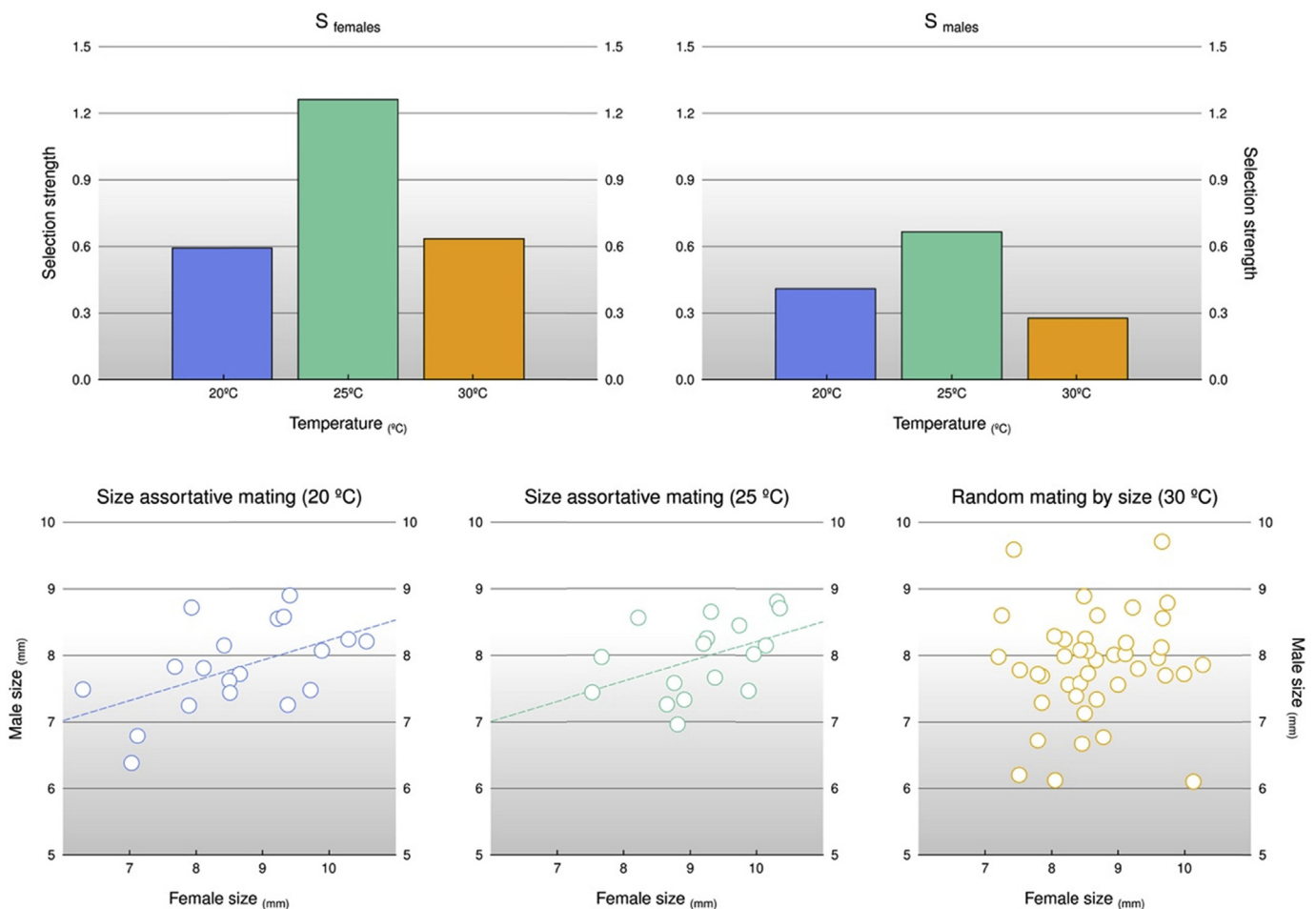
For all three tested temperatures, the selection strength on body size (Fig. 3, top panels) was high in females, indicating that males primarily target larger mating partners, which have the largest brood sacs and are able to carry more eggs. When considering the size of mating and non-mating females, a factorial ANOVA showed a significant interaction between reproductive status and temperature ( $F(2,178) = 4.140$ ,  $P < 0.05$ ), with the mating females, at 25 °C, being significantly larger (25 °C = 9.293 mm; average body length) than all other female groups (Newman-Keuls post hoc test). Post-hoc tests also revealed that the reproducing females at 20 °C and 30 °C were smaller but of comparable sizes (20 °C = 8.629 mm; 30 °C = 8.646 mm). Non-mating females at all three tested temperatures showed similarly small body sizes (20 °C = 7.210 mm; 25 °C = 6.761 mm; 30 °C = 6.801 mm). For males, no significant interaction was observed between mating status and temperature ( $F(2,177) = 1.050$ ,  $P = 0.351$ ). Nevertheless, differences in body length were observed between the larger mating males and the smaller non-mating males ( $F(1,177) = 24.020$ ,  $P < 0.05$ ).

As swimming speed may potentially impact couple formation (e.g., slower females may be more easily grabbed by males and have more difficulty releasing from less preferred mates), we looked into the relationship between *A. franciscana* size and swimming speed. Positive correlations between body length and swimming speed were only observed for females at 20 °C and 25 °C (20 °C:  $y = 0.487x - 2.360$ ,  $R^2 = 0.694$ ,  $P < 0.05$ ; 25 °C:  $y = 0.416x - 1.214$ ,  $R^2 = 0.510$ ,  $P < 0.005$ ), and males at 25 °C ( $y = 0.402x - 1.226$ ,  $R^2 = 0.581$ ,  $P < 0.01$ ). Furthermore, males and females at 25 °C showed identical slopes ( $F(1,24) = 0.006$ ,  $P = 0.937$ ) and elevations ( $F(1,25) = 0.443$ ,  $P = 0.512$ ), meaning that males and females of equivalent sizes, at this temperature, swim at similar speeds. In the remaining groups (females at 30 °C; males at 20 °C and 30 °C), no significant correlation between size and swimming speed was observed. An ANOVA conducted on female and male swimming speeds, at the three tested temperatures, revealed no significant interaction (sex and temperature;  $F(2,72) = 1.019$ ,  $P = 0.366$ ) nor differences within each considered factor (sex:  $F(1,72) = 0.059$ ,  $P = 0.830$ ; temperature:  $F(2,72) = 6.469$ ,  $P = 0.134$ ). Results are similar when controlling for body length.

Our simulations, based on the swimming speeds of males and females, show that the probability of size assortative mating occurring



**Fig. 2.** Examples of the relationship between *Artemia franciscana* body length and traits possibly related with reproductive output, at three temperatures (20 °C, 25 °C and 30 °C). Side images show how measurements were conducted (OD = interorbital distance; BA = brood sac area; AW = antenna width; AL = antenna length; LT = body length).



**Fig. 3.** Selection strength on body size in *A. franciscana* (top panels) and patterns of male and female mating patterns (bottom panels).



independently from mate choice is small. At 20 °C and 30 °C, the probability of observing a size assortative mating pattern dependent on swimming speed alone is very low ( $6.520\% \pm 0.694$  and  $5.750\% \pm 0.427$ , respectively). At 25 °C, while the probability increases ( $16.100\% \pm 0.891$ ), the prospects of mate choice-independent size assortative mating are still meagre.

#### 4. Discussion

*Artemia* growth and survival, from nauplius to the adult stage, are life history characteristics well-known to vary with temperature (Browne and Wanigasekera, 2000). Our observations supported this relationship, with sexual maturity occurring sooner and mortality rates increasing with higher temperatures. It is reasonable to hypothesise that mortality rates relate to the fact that rising temperatures impose additional developmental stress, by speeding metabolism. Irwin et al. (2007) showed, in *A. franciscana*, that temperature was the main factor affecting oxygen consumption, whose rate significantly expanded as water temperatures increased.

Given the temperature sensitivity of growth rate, especially noticeable during *A. franciscana* early life stages (Clarke, 2017), we were able to promptly detect differences in growth (body length) between the tested temperatures. As all individuals derived from the same batch of cysts, the temperature-dependent difference in growth rate, which correlates with the observed disparities in the onset of sexual maturity, effectively highlights the extent of *A. franciscana* developmental plasticity. Although temperature elicited distinct developmental speeds, trait growth rates were largely similar and independent from water temperature. Specifically, sexual traits, such as male secondary antennae or female brood pouches, grew at similar speeds at all tested water temperatures (i.e., higher or lower temperatures did not differentially impact the expression of morphological traits associated with reproduction).

Given the species' intrinsic ability to adaptively respond within broad temperature intervals, it could seem that a tentative prediction of the evolutionary consequences imposed by directional changes in abiotic variables, such as those predicted by climate change, would be hard to grasp. Based on our results, a reasonable scenario could be put forward where increasingly higher water temperatures (but still within the species physiological thermal limits) would progressively shorten intergenerational intervals (as sexual maturity occurs sooner at higher temperatures) while average adult size contracted (following the classic temperature-size response). At least in the short term, even for a closed population, these changes would not necessarily require impacts in the genome as the genetic basis of local adaptation seems to be in place. Despite higher mortalities, unless males and females are differentially affected by temperature and fitness is impacted, one can imagine that *A. franciscana* phenotypic plasticity can potentially provide sufficient buffer for the effects of incremental temperature regimes, at least in the short term.

Sexual selection is as a powerful evolutionary force able to greatly influence genome evolution. As such, disruptions to a population mating system can result in unpredictable consequences, theoretically even within short time intervals (Rode et al., 2011). Our temperature challenge experiments showed that, close to the optimal developmental temperature (Browne et al., 1988), *A. franciscana* mating is size assortative (Arnqvist et al., 1996; Mobley et al., 2013). By choosing larger females (with larger brood pouches), males should be able to maximize their reproductive success. Sexual selection strength calculated for our experimental populations confirms that males clearly select larger females as preferential mating partners. Preferred females, able to choose (Tapia et al., 2015), should also prefer mating with large males more readily than smaller ones (Forbes et al., 1992). Females could be seeking direct benefits in the form of increased swimming speed during riding position that allows for better nourishment or indirect genetic benefits for the offspring. Tapia et al. (2015) described female

behaviors that allow for male rejection (energetic movements to interrupt the amplexus), showing that females are far from passive bystanders. The calculated selection strength confirms that females tend to mate with the larger males.

When our experimental *A. franciscana* populations experienced temperatures that deviate from the optimum, we were able to notice alterations in mating patterns. Changes were subtler at lower temperatures, with individuals still mating size assortatively. As swimming speed alone seems to be an unlikely justification for the establishment of size assortative mating, we hypothesise that, at lower temperatures, intrasexual competition and/or mate choice patterns might be relaxed, but still in place. Indeed, selection intensity at 20 °C was significantly reduced for females (males still preferentially mated with the larger females, but less markedly than at 25 °C) but not for males. While swimming speed was associated with body size in females, the lack of this correlation in males (unlike what was observed at 25 °C) could mean that females of any size can be approached and touched (see Tapia et al., 2015) by a phenotypically more diverse group of males (and not only those of similar size and speed). Male harassment is known to be potentially able to reduce female fitness (Ojanguren and Magurran, 2007).

The most noticeable impacts on the mating system were registered when temperatures rose above the species' optimum. The typical size assortative mating pattern disappeared. As selection strength for females did not diverge from that registered at the lower tested temperature, we believe that, although males were still preferentially choosing the larger mating partners, females may have been unwilling to dislodge less preferable males, accepting amplexus from smaller males as well. Our results highlight a disruption of *A. franciscana* mating system, especially pronounced under high temperatures. This alteration in mating pattern involves a sensible relaxation of sexual selection intensity as reproduction becomes progressively more random.

Seasonal variation in sexual selection intensity is not uncommon, as mate choice and intersexual competition can significantly vary from the onset to the end of the breeding season (Cunha et al., 2014). This is expected as, in ectothermic aquatic organisms, the expression of reproduction-related behaviours often correlates with temperature. For instance, in wild populations of the pipefish *Syngnathus abaster*, the higher water temperatures occurring towards the end of the breeding season trigger a relaxation of sexual selection intensity (Silva et al., 2007; Cunha et al., 2014). Furthermore, if these pipefish are directly exposed to high temperatures (i.e., without experiencing the typically milder temperatures usually occurring at the onset of the breeding season, when sexual selection is high), mate choice and competition are impacted, instantly becoming less stringent (Silva et al., 2007).

Zapata et al. (1990) showed an association between heterozygosity and male mating success in *A. franciscana*. A temperature-dependent increase in female willingness to mate with *a priori* less desirable males (i.e., weakened mate choice) can thus diminish the usefulness of male sexually selected traits. Although common in nature, the effects of relaxed selection on trait loss have been understudied (Lahti et al., 2009). Nevertheless, we can hypothesise that a reduction in selection intensity under new temperature conditions can make the expression of sexually selected traits ultimately maladaptive.

It is commonly accepted that directional selection assumes a key role in driving adaptation and evolutionary change (Hoekstra et al., 2001). Nevertheless, the impacts of temperature regimes on sexual selection have received little attention (Monteiro et al., 2017). As climate change is an inescapable reality, understanding species or population responses to progressively higher temperatures is paramount. Our results confirm how anthropogenic disturbances (e.g., temperature) are able to affect selection pressures, with potential longstanding effects on the viability and evolution of populations.

## Conflicts of interest

None.

## Acknowledgments

We would like to thank Kenyon Mobley for his helpful comments and text revision, and acknowledge the contributions of two anonymous reviewers. Nuno Monteiro was funded by the Portuguese Foundation for Science and Technology through a postdoc grant (SFRH/BPD/103829/2014).

## References

- Allen, S.L., Bonduriansky, R., Sgro, C.M., Chenoweth, S.F., 2017. Sex-biased transcriptome divergence along a latitudinal gradient. *Mol. Ecol.* 26, 1256–1272.
- Amat, F., 1980. Diferenciación y distribución de las poblaciones de *Artemia* (Crustáceo, Branquiópodo) de España. I. Análisis morfológico. *Estudios alométricos referidos al crecimiento y a la forma. Investig. Pesq.* 44, 217–240.
- Amat, F., Hontoria, F., Navarro, J.C., Vieira, N., Mura, G., 2007. Biodiversity loss in the genus *Artemia* in the Western Mediterranean region. *Limnética* 26, 387–404.
- Amat, F., Hontoria, F., Ruiz, O., Green, A., Sánchez, M., Figuerola, J., Hortas, F., 2005. The American brine shrimp as an exotic invasive species in the western Mediterranean. *Biol. Invasions* 7, 37–47.
- Anufrieva, E.V., Shadrin, N.V., 2014. The swimming behavior of *Artemia* (Anostraca): new experimental and observational data. *Zoology* 117, 415–421.
- Arnqvist, G., Rowe, L., Krupa, J.J., Sih, A., 1996. Assortative mating by size: a meta-analysis of mating patterns in water striders. *Evol. Ecol.* 10.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., Courchamp, F., 2012. Impacts of climate change on the future of biodiversity. *Ecol. Lett.* 15, 365–377.
- Browne, R.A., Wanigasekera, G., 2000. Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. *J. Exp. Mar. Biol. Ecol.* 244, 29–44.
- Browne, R.A., Davis, L.E., Sallee, S.E., 1988. Effects of temperature and relative fitness of sexual and asexual brine shrimp *Artemia*. *J. Exp. Mar. Biol. Ecol.* 124, 1–20.
- Candolin, U., Heuschele, J., 2008. Is sexual selection beneficial during adaptation to environmental change? *Trends Ecol. Evol.* 23, 446–452.
- Clarke, A., 2017. *Principles of Thermal Ecology: Temperature, Energy, and Life*. Oxford University Press, New York.
- Criel, G.R.J., Macrae, T., 2002. Reproductive biology of *Artemia*. In: Abatzopoulos, T.J., Beardmore, J., Clegg, J.S., Sorgeloos, P. (Eds.), *Artemia: Basic and Applied Biology*. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 39–128.
- Cunha, M., Berglund, A., Monteiro, N.M., 2014. The intrinsically dynamic nature of mating patterns and sexual selection. *Environ. Biol. Fish.* 1047–1058.
- Forbes, M.R., Pagola, H., Baker, R.L., 1992. Causes of a non-random pairing by size in the brine shrimp, *Artemia salina*: (Crustacea: Anostraca). *Oecologia* 91, 214–219.
- Fujimoto, S., Miyake, T., Yamahira, K., 2015. Latitudinal variation in male competitiveness and female choosiness in a fish: are sexual selection pressures stronger at lower latitudes? *Evol. Biol.* 42, 75–87.
- Grazer, V.M., Martin, O.Y., 2012. Investigating climate change and reproduction: experimental tools from evolutionary biology. *Biology* 1, 411–438.
- Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hoang, A., Hill, C.E., Beerli, P., Kingsolver, J.G., 2001. Strength and tempo of directional selection in the wild. *Proc. Natl. Acad. Sci. U. S. A.* 98, 9157–9160.
- Hoffmann, A.A., Sgro, C.M., 2011. Climate change and evolutionary adaptation. *Nature* 470, 479–485.
- Irwin, S., Wall, V., Davenport, J., 2007. Measurement of temperature and salinity effects on oxygen consumption of *Artemia franciscana* K., measured using fibre-optic oxygen microsenors. *Hydrobiologia* 575, 109–115.
- Lahti, D., Johnson, N., Ajie, B., Otto, S., Hendry, A., Blumstein, D., Coss, R., Donohue, K., Foster, S., 2009. Relaxed selection in the wild. *Trends Ecol. Evol.* 24, 487–496.
- Lenz, P.H., Browne, R.A., 1991. *Ecology of Artemia*. In: Browne, R.A., Sorgeloos, P., Trotman, C.N.A. (Eds.), *Artemia Biology*. CRC Press, Boca Raton, Florida, pp. 237–253.
- Lindman, H.R., 1974. *Analysis of Variance in Complex Experimental Designs*. W H Freeman, San Francisco.
- Medina, G.R., Goenaga, J., Hontoria, F., Cohen, G., Amat, F., 2007. Effects of temperature and salinity on prereproductive life span and reproductive traits of two species of *Artemia* (Branchiopoda, Anostraca) from Argentina: *Artemia franciscana* and *A. peruviana*. *Hydrobiologia* 579, 41–53.
- Mobley, K., Chakra, M.A., Jones, A.G., 2013. No evidence for size-assortative mating in the wild despite mutual mate choice in sex-role-reversed pipefishes. *Ecol. Evol.* 4, 67–78.
- Monteiro, N., Cunha, M., Ferreira, L., Vieira, N., Antunes, A., Lyons, D., Jones, A.G., 2017. Parabolic variation in sexual selection intensity across the range of a cold-water pipefish: implications for susceptibility to climate change. *Global Change Biol.* 23, 3600–3609.
- Monteiro, N.M., Lyons, D.O., 2012. Stronger sexual selection in warmer waters: the case of a sex role reversed pipefish. *PLoS One* 7.
- Ojanguren, A.F., Magurran, A.E., 2007. Male harassment reduces short-term female fitness in guppies. *Behaviour* 144, 503–514.
- Parnesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421, 37–42.
- Rode, N.O., Charmantier, A., Lenormand, T., 2011. Male-female coevolution in the wild: evidence from a time series in *Artemia franciscana*. *Evolution* 65, 2881–2892.
- Rodrigues, C.M., Bio, A.M., Amat, F.D., Monteiro, N.M., Vieira, N.M., 2012. Surviving an invasion: characterization of one of the last refugia for *Artemia* diploid parthenogenetic strains. *Wetlands* 32, 1079–1090.
- Shuster, S.M., Wade, M.J., 2003. *Mating Systems and Strategies*. Princeton University Press, Princeton, New Jersey.
- Silva, K., Vieira, M.N., Almada, V.C., Monteiro, N.M., 2007. The effect of temperature on mate preferences and female-female interactions in *Syngnathus abaster*. *Anim. Behav.* 74, 1525–1533.
- Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J. Exp. Biol.* 213, 912–920.
- Sprouffske, K., Wagner, A., 2016. Growthcurver: an R package for obtaining interpretable metrics from microbial growth curves. *BMC Bioinf.* 17, 172.
- Tapia, C., Parra, L., Pacheco, B., Palma, R., Gajardo, G., Quiroz, A., 2015. Courtship behavior and potential indications for chemical communication in *Artemia franciscana* (Kellogg 1906). *Gayana* 79, 152–160.
- Xiaoguo, J., Wu, J., Chen, Z., Fengxiang, L., 2009. Effects of temperature on courtship and copulatory behaviours of a wolf spider *Pardosa astrigera* (Araneae: Lycosidae). *J. Therm. Biol.* 34, 348–352.
- Zapata, C., Gajardo, G., Beardmore, J.A., 1990. Multilocus heterozygosity and sexual selection in the brine shrimp *Artemia franciscana*. *Mar. Ecol. Prog. Ser.* 62, 211–217.